

## The CD40/CD40L axis in glioma progression and therapy

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See the article by Chonan et al., on pages 1453–1462.

Generation or enhancement of immune responses through engagement of costimulatory receptors such as CD40 ligation is an essential component of antitumor immunity and could be exploited in glioma immunotherapy. Nevertheless, the importance of adequate immune stimulation is somewhat overshadowed by the current rush towards blocking immune inhibition using checkpoint-blockade antibodies. Therefore, the current study by Chonan et al.<sup>1</sup> in which CD40/CD40L expression and manipulation are revisited in glioma is a useful reminder of the stimulatory component of antiglioma immunity.

The best understood role of CD40/CD40L is in immune cell interactions. The CD40 glycoprotein and its ligand CD40L (CD154) are members of the tumor necrosis factor (TNF) and TNF receptor superfamily. CD40 is expressed on dendritic cells, B cells, and macrophages; upon ligation with CD40L expressed by activated CD4T cells, the antigen-presenting function of these cell types is enhanced, inflammatory mediators are released, and B cells differentiate towards plasma cells or memory cells.<sup>2</sup> Moreover, these immunostimulatory effects can be recapitulated using agonistic anti-CD40 antibodies. However, exploiting this pathway is complex because of different downstream consequences after CD40 ligation according to cell type and context. In fact, expression of CD40 occurs not only on the key immune cells listed above but also on non-immune cells including endothelial cells, epithelial cells, and platelets.<sup>2</sup> Similarly, CD40L has a wide expression pattern with expression noted by platelets, endothelial cells, fibroblasts, and muscle cells, particularly in the context of inflammation.<sup>3</sup>

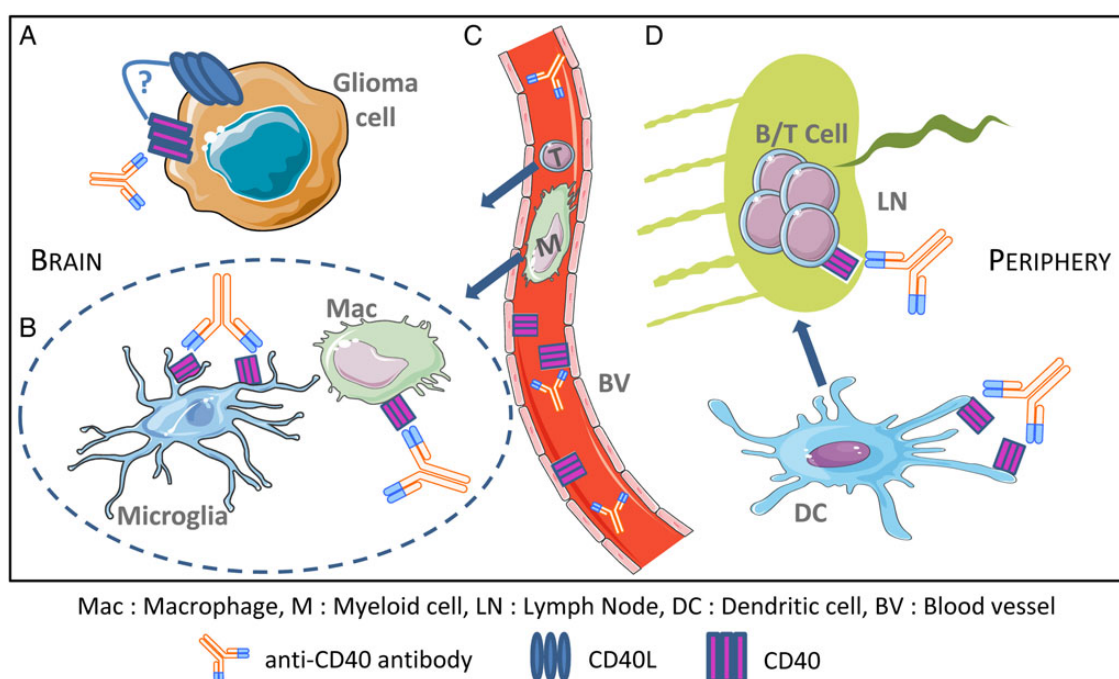
In the case of malignancy, expression and function of CD40 and CD40L on the tumor cell enlarge the potential interactions and consequences. Indeed, tumor cell expression of CD40 is quite frequent, with most hematological malignancies expressing CD40, as well as expression being reported in melanoma, multiple carcinomas (reviewed by Vonderheide<sup>4</sup>) and a few studies concerning glioma.<sup>1,5,6</sup> Chonan et al.<sup>1</sup> made the intriguing observations that CD40 is more highly expressed by grade III gliomas than grade IV glioblastoma, that there is also coexpression of CD40L by glioma (Fig. 1), and that higher expression of CD40/CD40L (by quantitative PCR) is a positive prognostic indicator of survival. These data raise the issues of CD40/CD40L function in cancer cells and whether there are some particularities in

glioma. Ligation of CD40 on tumor cells may have somewhat different outcomes than normal immune cells, with proapoptotic signaling reported for various lymphoid and solid tumors as well as protumoral effects in low-grade B-cell malignancies.<sup>4</sup> Furthermore, CD40/CD40L coexpression was a negative prognostic factor in malignant melanoma.<sup>7</sup> Concerning glioma, CD40 ligation was reported to either induce secretion of VEGF<sup>6</sup> or induce no signaling at all.<sup>5</sup> A major issue is that cell-surface expression of CD40 may be insufficient for a biological effect, as was observed for in vitro-cultured glioma lines.<sup>5</sup> Expression in vivo can be difficult to assess, particularly if biopsies are analyzed by reverse transcription-PCR or Western blot since many nontumoral cells express both CD40 and CD40L. It is noteworthy that glioma expression of CD40 was mainly cytoplasmic by immunohistochemistry,<sup>6</sup> making any causative association with prognosis difficult to justify. Regarding the novel finding of CD40L expression in glioma and its positive correlation with prognosis,<sup>1</sup> it is conceivable that this could impact tumor cell interactions with CD40-expressing immune cells infiltrating the tumor bed, but this remains to be determined.

In contrast to the speculative roles of CD40/CD40L expressed by glioma cells on disease progression, there is considerable interest for therapeutically enhancing antitumor immunity by administering CD40 ligands (antibodies or recombinant CD40L). Currently, 4 anti-CD40 antibodies are being explored clinically for certain cancer indications, with different outcomes expected depending upon the epitope targeted, the isotype, and whether the antibody is antagonistic or agonistic.<sup>3,8</sup> Moreover, CD40 ligation was previously validated in the context of combination therapies in mouse models for glioma.<sup>9,10</sup> Chonan et al.<sup>1</sup> used an agonistic anti-CD40 antibody in combination with a tumor lysate-based vaccine (in some cases with dendritic cells) to treat mice bearing orthotopic GL261 gliomas or NSCL61 glioma-initiating cell (GIC)-like tumors (Fig. 1); combination immunotherapy provided a major therapeutic effect for GL261 but modest effect for the GIC tumor. For the latter, addition immunostimulation with another immunostimulatory antibody (against OX40) was required. It would be interesting to know whether, in these models, sufficient anti-CD40 antibody reached the tumor bed to ligate CD40 on the glioma cells or on the pre-existing immune infiltrate, but

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**Fig. 1.** Expression of CD40/CD40L in glioma and potential interactions with agonistic anti-CD40 antibody. (A) Proposed coexpression of CD40 and CD40L by glioma cells<sup>1</sup> and hypothetical autocrine stimulation or stimulation by anti-CD40 antibody. (B) Immune cells targeted by anti-CD40 antibody in glioma microenvironment. (C) CD40-expressing endothelial cells and T cell/myeloid cell extravasation to brain. (D) Key role of anti-CD40 ligation on dendritic cells and subsequent B cell/T cell activation in the periphery (lymph nodes/spleen) in the context of therapeutic glioma vaccination.

this was not addressed. Both local effects (increased apoptosis, reduced proliferation, and T cell infiltration) as well as systemic effects (lymphocyte activation in the spleen) were observed, but these effects would be compatible with the antibody interacting with immune cells extracranially. One major mechanism contributing to glioma progression is the balance and function of myeloid cell populations.<sup>11,12</sup> In this regard, agonistic anti-CD40 antibodies can exert antitumor effects through myeloid cell activation or repolarization, as demonstrated clinically in pancreatic carcinoma,<sup>13</sup> and in combination with COX-2 inhibition in mouse glioma models.<sup>9</sup>

Targeting CD40 for glioma therapy merits continued investigation, although arguably the issue of tumor cell expression of CD40/CD40L is not a major concern for immunotherapy. Since glioma, and glioblastoma in particular, is a poorly immunogenic and highly immunosuppressive tumor, successful immunotherapy will need us to “press the gas pedal” (immunostimulation) as well as “release the brakes” (checkpoint blockade).<sup>14</sup> Clinical tools in the form of new generation glioma vaccines and human or humanized agonistic and antagonistic antibodies are now available for clinical trials. The challenge now is to construct appropriate combinations and to walk the tightrope of therapeutic potency and the inevitable toxicities.

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## References

- Chonan M, Saito R, Shoji T, et al. CD40/CD40L expression correlates with the survival of patients with glioblastomas and an augmentation in CD40 signaling enhances the efficacy of vaccinations against glioma models. *Neuro Oncol.* 2015;17(11):1453–1462.
- Quezada SA, Jarvinen LZ, Lind EF, et al. CD40/CD154 interactions at the interface of tolerance and immunity. *Ann Rev Immunol.* 2004;22:307–328.
- Hassan GS, Stagg J, Mourad W. Role of CD154 in cancer pathogenesis and immunotherapy. *Cancer Treat Rev.* 2015;41(5):431–440.
- Vonderheide RH. Prospect of targeting the CD40 pathway for cancer therapy. *Clin Cancer Res.* 2007;13(4):1083–1088.
- Wischhusen J, Schneider D, Mittelbronn M, et al. Death receptor-mediated apoptosis in human malignant glioma cells: modulation by the CD40/CD40L system. *J Neuroimmunol.* 2005;162(1–2):28–42.
- Xie F, Shi Q, Wang Q, et al. CD40 is a regulator for vascular endothelial growth factor in the tumor microenvironment of glioma. *J Neuroimmunol.* 2010;222(1–2):62–69.
- van den Oord JJ, Maes A, Stas M, et al. CD40 is a prognostic marker in primary cutaneous malignant melanoma. *Am J Pathol.* 1996;149(6):1953–1961.
- Hassan SB, Sorensen JF, Olsen BN, et al. Anti-CD40-mediated cancer immunotherapy: an update of recent and ongoing clinical trials. *Immunopharmacol Immunotoxicol.* 2014;36(2):96–104.
- Kosaka A, Ohkuri T, Okada H. Combination of an agonistic anti-CD40 monoclonal antibody and the COX-2 inhibitor

- celecoxib induces anti-glioma effects by promotion of type-1 immunity in myeloid cells and T-cells. *Cancer Immunol Immunother.* 2014;63(8):847–857.
10. Derouazi M, Di Berardino-Besson W, Belnoue E, et al. Novel cell-penetrating peptide-based vaccine induces robust CD4<sup>+</sup> and CD8<sup>+</sup> T Cell-mediated antitumor immunity. *Cancer Res.* 2015; (Jun 26 doi:10.1158/0008-5472.CAN-14-3017).
11. Riccadonna C, Walker PR. Macrophages and microglia in brain malignancies. In: Biswas SK, ed. *Tumor Microenvironment and Myelomonocytic Cells*. Rijeka, Croatia: InTech; 2012:173–2200.
12. Pyonteck SM, Akkari L, Schuhmacher AJ, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med.* 2013;19(10):1264–1272.
13. Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science.* 2011;331(6024):1612–1616.
14. Melero I, Grimaldi AM, Perez-Gracia JL, et al. Clinical development of immunostimulatory monoclonal antibodies and opportunities for combination. *Clin Cancer Res.* 2013;19(5): 997–1008.